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PROCEEDINGS
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Vol. XXVII SECTION-B Part VI

ON A NEW SPECIES OF THE GENUS *EUMEGACETES* LOOSS, 1900

(Family Lecithodendriidae Odhner, 1911)

By

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(Received on 13th November 1957)

Eumegacetes riparius sp. nov.

A single specimen of this trematode was obtained from the cloaca of a Dusky Crag-Martin, *Riparia concolor* Sykes, shot by the author at the outskirts of Anupshahr (District Bulandshahr, U. P.)

The body (Fig. 1) is elongated and somewhat oval in shape with a rounded anterior and a bluntly pointed posterior end. It measures 2·849 mm.×1·36 mm. The maximum width of the body occurs in the testicular region. The body cuticle is armed with minute backwardly directed spines. The suckers are large with well developed musculature. The oral sucker is subterminal and measures 0·571 mm.× 0·624 mm. The ventral sucker is equatorial in position and measures 0·645 mm.× 0·579 mm.

A prepharynx is absent. The mouth leads into a large pharynx which is much broader than long measuring 0·129 mm.×0·31 mm. Oesophagus is absent.

The intestinal caeca pass, at first transversely lateralwards but near the lateral sides of the body these bend backwards and continue upto a little in front of the posterior end of body. Throughout most of their course the intestinal caeca are partly overlapped by the vitelline follicles and the coils of the uterus.

The testes are symmetrically situated in the intercaecal region between the pharynx and the ventral sucker. The right testis is almost spherical in shape and is somewhat larger than the left one. It measures 0·365 mm.×0·324 mm. The left testis is obliquely oval in shape and measures 0·324 mm.×0·279 mm. The cirrus sac is somewhat dextrally situated between the pharynx and the ventral sucker. Due to a concavity on its inner side, the cirrus sac appears to be slightly crescent-shaped. It measures 0·459 mm.×0·178 mm. its maximum width being in its proximal half. The seminal vesicle is quite large and is strongly bent in the form of a U. The pars prostatica is long, distally coiled and is surrounded with thickly set unicellular prostatic gland cells. It terminates into a short ductus ejaculatorius. The genital pore is situated just behind the pharynx and is slightly displaced to the right side of the median line of body.

The ovary is dextrally situated close behind the ventral sucker. It is oval in shape with its long axis lying obliquely to that of the body. It measures 0·278 mm.×0·228 mm. The Mehli's gland is median in level with the ovary. The receptaculum seminis is somewhat pear-shaped. It is obliquely situated between the ovary and the ventral sucker. It measures 0·152 mm.×0·07 mm. The uterus is extensive and highly coiled. It fills the post-ovarian part of the body and also extends anteriorly along both lateral sides of the body upto the testicular region. The lateral ascending and descending arms of the uterus are intermingled with the vitelline follicles. The vitellaria are characteristically composed of large, oval to pyriform follicles which, at places, tend to aggregate in rosette-shaped groups. Anteriorly, the vitelline follicles extend upto the level of the pharynx while posteriorly they extend almost upto the posterior end of body. Throughout their course, the vitelline follicles partly overlap the intestinal caeca. The transverse vitelline ducts arise at the level of the ventral sucker and run obliquely backwards and inwards. At the level of the ovary, the two transverse vitelline ducts unite medially to form a small vitelline reservoir. The eggs are numerous, yellowish in colour, operculated and somewhat pointed at the opercular pole. The eggs measure 0·021 mm.×0·0258 mm.

The excretory bladder is Y-shaped. The excretory cornua are distinctly divided into small chambers and extend upto the level of the oral sucker.

DISCUSSION

The present form can be sharply distinguished from all the species of the genus *Eumegacetes* Looss, 1900, hitherto known, by the presence of body spines, by the large size and characteristically oval to pyriform shape of the vitelline follicles and by the chambered nature of the excretory cornua. Further, the cirrus sac, in the present form, lies immediately in front of the ventral sucker whereas in all other species of this genus, the cirrus sac is situated considerably in front of the ventral sucker.

In view of these differences, it is evident that the present form is a new species for which the name *Eumegacetes riparius* is, here, proposed.

As far as the author can determine, the genus *Eumegacetes* Looss, 1900 includes only nine species including the presently described one. A key for the identification of all known species of this genus is given as follows :—

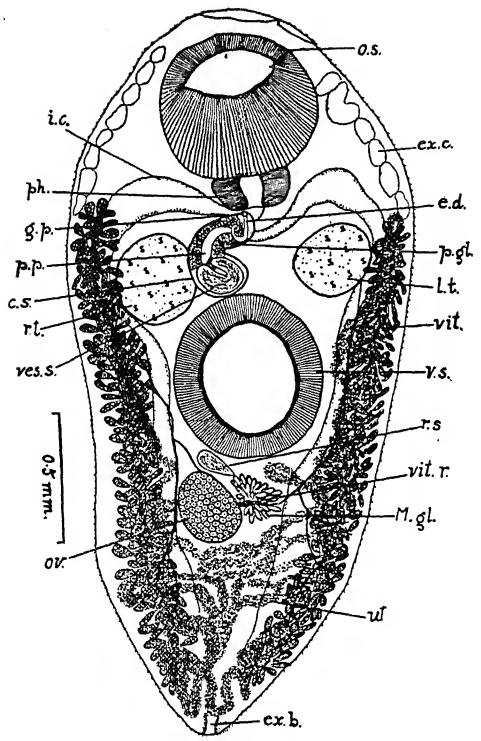

Fig. 1. *Eumegacetes riparius sp. nov.*, (ventral view).

c.s.—cirrus sac; e.d.—ejaculatory duct; ex.b.—excretory bladder; ex.c.—excretory cornu; g.p.—genital pore; l.c.—intestinal caecum; l.t.—left testis; M.gl.—Mehli's gland; o.s.—oral sucker; ov.—ovary; p.gl.—prostatic gland; ph.—pharynx; p.p.—prostatic duct; r.s.—receptaculum seminis; r.t.—right testis; ut.—uterus; ves. s.—vesicula seminalis; vit.—vitellaria; vit. r.—vitelline reservoir; v.s.—ventral sucker.

A KEY TO THE SPECIES OF THE GENUS EUMEGACETES LOOSS, 1900

1.	Vitelline follicles extend anteriorly upto the testes or a little in front of them.	..... 2.
	Vitelline follicles never extend anteriorly upto the testes.	..... 6.
2.	Testes equatorial; ovary far behind the ventral sucker near the posterior end of body.	.....*E. perodiosus.* Travassos, 1922.
	Testes pre-equatorial; ovary only slightly behind ventral sucker and considerably in front of the posterior end of body.	..... 3.
3.	Body spinose; excretory cornua chambered.	.....*E. riparius.* Sp. nov.
	Body aspinose; excretory cornua not chambered.	..... 4.
4.	Cirrus sac long, coiled on itself.	.....*E. microdiosus.* Chauhan, 1940.
	Cirrus sac much swollen at base and crescent shaped anteriorly.	.....*E. artamii.* Mehra, 1935.
	Cirrus sac somewhat U-shaped ...	..... 5.
5.	Vitelline follicles extend in front of the testes; genital pore at base of the pharynx.	.....*E. mehraii.* Jha, 1943.
	Vitelline follicles never extend in front of the middle of testes; genital pore behind the pharynx.	.....*E. emendatus.* Braun, 1901.
(1)6.	Ventral sucker equatorial; vitelline follicles never extend in front of the posterior margin of ventral sucker.	.....*E. contribulans.* Braun, 1901.
	Ventral sucker post-equatorial; vitelline follicles extend in front of the posterior margin of ventral sucker.	..... 7.
7.	Ovary near the posterior end of body ...	.....*E. braunii.* Mehra, 1935.
	Ovary immediately behind the ventral sucker.	*E. medioximus.* Braun, 1901.

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STUDIES ON A NEW SPECIES OF THE GENUS *PATAGIFER* DIETZ, 1909 (TREMATODA : ECHINOSTOMATIDAE)

By

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(Received on December 9, 1957)

INTRODUCTION

Twenty worms were collected from the intestine of white ibis, *Threskiornis melanocephalus* shot neat Raipur in the month of November, 1956. They belong to the genus *Patagifer* Dietz, 1909. Four species of the genus are known from India. The present form shows several contrasting features from previously described species of the genus and necessitates the creation of a new species.

PATAGIFER SARAI, N. SP.

The worm is cream coloured, ribbon like, elongated measuring 20·0—23·2 mm. in length and 2·1 – 2·58 mm. in maximum breadth at the region of the acetabulum. At the anterior end it possesses a deeply bilobed collar. Each lobe of the collar has a single row of spines. The collar is 1·5 – 1·86 mm. long and 2·02 – 2·36 mm. broad.

The study of collar spines and their measurements are from a specimen cleared in glycerine. There are 30 collar spines on each side including the angular spines. The marginal spines at the anterior region are bluntly pointed. They become gradually more blunt towards the posterior region of the collar where they become rod shaped with blunt ends. The first spine is more or less hidden. The second spine of the right collar lappet measures 0·056 × 0·028 mm. The spines gradually increase in size till in the ventrolateral region we get comparatively bigger marginal spines – the 19th, the 20th and the 21st spines which are directed outwards. The 19th. spine of the right lappet measures 0·129 × 0·045 mm. and the 21st. spine of the same side measures 0·135 × 0·048 mm. There is again gradual reduction in size of the spines till 25th. marginal spine which is 0·09 × 0·039 mm. in size. The 26th. marginal spine is slightly bigger in size measuring 0·11 × 0·042 mm.

The angular spines constitute the group of four spines in two rows out of which two aborals are big in size. The inner and outer bigger angular spines of the right lappet measure 0·18 × 0·081 mm. and 0·156 × 0·066 mm. respectively. The inner and outer smaller angular spines of the same side measure 0·09 × 0·03 mm. and 0·114 × 0·03 mm. respectively. The collar spines of the left side correspond in position and size to those on the right side.

There are no spines on the general surface of the body. Just below the collar the body has a constriction which marks the neck region.

The oral sucker is subterminal and subspherical measuring 0·49 – 0·56 mm. × 0·43 × 0·45 mm. The ventral sucker is cupshaped and well developed. It lies at a distance of 1·72 – 2·45 mm. from the anterior notch of the head collar. It measures 1·98 – 2·06 mm. in length and 1·72 – 1·89 mm. in breadth.

The prepharynx is absent. The pharynx is muscular, oval in shape and measures 0·18 – 0·31 mm. × 0·28 – 0·43 mm. in size. It is smaller than the oral sucker. The oesophagus is 0·95 – 1·16 mm. long and 0·042 mm. – 0·056 mm. broad. The intestinal bifurcation lies at a distance of 1·32 – 1·83 mm. from the anterior collar notch. The intestinal caeca lie in the lateral regions of the body extending almost to the posterior extremity of the worm. The caeca are not crenated.

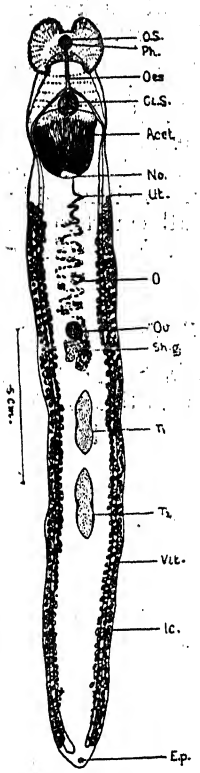
The gonads are located in the middle third of the body. The testes are elongated oval in shape with a constriction in the centre. In some worms the constriction in testes is more pronounced so as to give a bilobed appearance. They are placed one behind the other in the longitudinal axis, the distance between them being 0·43 – 0·47 mm. The anterior testis lies at a distance of 8·0 – 11·3 mm. from the anterior end. It measures 1·89 – 1·93 mm. × 0·6 × 0·77 mm. The posterior testis is 1·89 – 2·15 mm. × 0·60 mm. in size. The measurements show that the anterior and posterior testes are almost equal in size. The cirrus sac lies between the intestinal bifurcation and the acetabulum. It is partly overlapped by the ventral sucker. It measures 0·74 – 0·77 mm. × 0·53 – 0·64 mm. in size and contains a convoluted vesicula seminalis, pars prostatica, ductus ejaculatorious and a small cirrus. The genital pore is situated just behind the intestinal bifurcation in the median line.

The ovary is oval in shape and placed obliquely at a distance of 6·6 – 9·2 mm. from the anterior end. It lies at a distance of 0·9 – 1·5 mm. from the anterior testis. This distance is larger than the distance between the two testes. The ovary measures 0·56 – 0·6 mm. in length and 0·45 – 0·49 mm. in breadth. The oviduct arises from the posterior region of the ovary. The shell gland lies posterior to the ovary. The tubular receptaculum seminis is present which is partly hidden in the shell gland mass. The transverse vitelline ducts lie between the anterior testis and the ovary. They meet in the median line and give rise to a small common vitelline duct which opens into the ootype. The uterus arises from the posterior margin of the ootype, travels above the ovary and coils transversely in the intercaecal area between the acetabulum and the ovary. The uterine eggs are oval and measure 0·081 – 0·093 × 0·054 mm. The vitellaria are follicular and extend from the posterior margin of the acetabulum to the posterior end of body occupying the lateral regions covering the intestinal caeca.

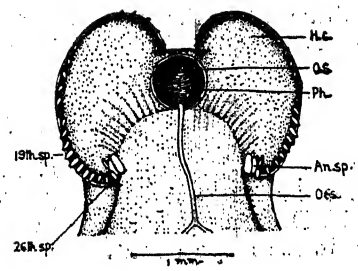
The excretory pore is subterminal at the posterior end and leads into a Y shaped bladder as in other species.

DISCUSSION

There are nine species of the genus *Patagifer* reported so far and of these four are known from India. They are *P. wesleyi* Verma, 1936, *P. Simarai* Nigam, 1944, *P. chandrapuri* Srivastava, 1952 and *P. srivastavai* Peter, 1954. The other species described are *P. bilobus* (Rud. 1819) Dietz, 1910; *P. consimilis* Dietz, 1909, *P. accuminatus* Johnston, 1916; *P. fraternus* Johnston, 1916; and *P. parvispinosus* Yamaguti, 1933.



Text. fig. 1. *Patagifer sarai n. sp.*



Text. fig. 2. *Patagifer sarai* n. sp. Head Collar.

Acet. acetabulum; An. sp., angular spines, Ci. s. cirrus sac; Hc. Head Collar; I. C. intestinal caeca; NO. notch; O., eggs, Oes., oesophagus; O. S. oral sucker; Ov. ovary; Ph. Pharynx; Sh. g. shell gland; T_1., anterior testis; T_2 posterior testis; Ut., uterus; Vit., Vitelline glands; 19th sp., 19th collar spine; 26th. sp., 26th collar spine.

P. sarai, n. sp. differs from *P. simarai* in the following characters: collar not broader than the body, number of spines, testes larger and the cirrus sac only partly overlapped by the ventral sucker. It differs from *P. accuminatus* and *P. fraternus* by having the genital pore behind the intestinal bifurcation. *P. consimilis* and *P. bilobus* have less number of spines, the cirrus sac being almost wholly overlapped by the ventral sucker and in *P. consimilis* the vitellaria are branched. It differs from *P. parvispinosus* by having larger number of collar spines, different shape and size as well as position and arrangement of genital organs. The present form is separated from *P. wesleyi* and *P. chandrapuri* by the possession of notch in the posterior margin of the ventral sucker. The pharynx is larger than the oral sucker in *P. wesleyi* which is not so in the new species. It differs from *P. chandrapuri* in the following characters: less number of collar spines, the distance between the ovary and the anterior testis being larger than the distance between the two testes, the form of the testes, distribution of vitellaria and notched acetabulum. In *P. srivastavai* the intertesticular space is greater than the distance between the ovary and the anterior testis, the posterior testis is smaller than the anterior one, the oral sucker and the pharynx are of the same length and the number of collar spines on either side is 31. On account of the above differences the present form is allocated to a new species *Patagifer sarai*, n. sp. after the name of the village Saragaon from where the parasites were collected.

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NITROGEN NUTRITION OF *PHYLLOSTICTA ARTOCARPINA* (SYD ET BUTL)

By

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(Received on January 3, 1958)

Nitrogen is essential for the growth of the fungi. All the nitrogen sources are not equally suitable for different organisms. Numerous investigators have studied the role of nitrogen on the growth and reproduction of fungi, but our knowledge is not yet complete. Robbins (1937) and Steinberg (1950) have classified the fungi according to their ability to utilize different sources of nitrogen. Recently Subramanian and Srinivasapai (1953), Beckman *et al* (1953), Converse (1953), Hackskaylo *et al* (1954), Tandon and Bilgrami (1954 a, b) and Tandon and Grewal (1956) have reported different patterns of utilization of various nitrogen compounds by the fungi investigated by them.

Lilly and Barnett (1951) mentioned that a mixture of amino acids may or may not be utilized better than a single amino acid, because the effect of one amion acid on the utilization of another varies with the particular amino acid and the specific fungus used. Leonian and Lilly (1940) found that growth of *Phycomyces blakesleeanus* was better on a mixture of five amino acids than on their individual source.

Lindeberg (1944), Thom and Raper (1945), Norkrans (1950) and Tandon and Grewal (1956), have established that physiological specificity exists between two closely allied species of the same genus. It was, therefore, decided to undertake studies on the nitrogen requirements of *Phyllosticta artocarpina*, and compare with *P. cycadina* which had been investigated earlier (1954 a, b).

MATERIAL AND METHOD

The culture of *P. artocarpina* used in the earlier investigation (1957) was utilized again. Pyrex glassware and chemicals of highest purity were used. On the basis of previous resuls the pH of all the media was adjusted to 5·2 before autoclaving. For the study of the dry weight the fungus was inoculated into 25 ml liquid media dispensed in 150 ml conical flasks, plugged and sterilized as usual. These studies were conducted in two parts, in the first part KNO_3 of the basal medium (dextrose 5·0 gms, KH_2PO_4 1·75 gms, KNO_3 3·5 gms, $MgSO_4\,7\,H_2O$ 0·75 gm and double distilled water 1 litre) was replaced by 26 different sources of nitrogen compounds e.g., nitrate, nitrite, ammonia and organic nitrogen. The amount of nitrogen supplied in each case was equivalent to the quantity to the basal medium. The amount of peptone was similar to that of KNO_3. The inoculated flasks were incubated at 25°C for

15 days. After the incubation period the fungal mats were harvested and then dried at 65°C for two days. The dry weight was calculated by the usual method of the authors (1954 a). Four replicates of each series were used and their average values have been recorded. On the basis of statistical analysis, the dry weight results have been classified into 3 categories *viz.* good, moderate and poor. In the second part the rate of assimilation of 5 different amino acids and their mixture was studied chromatographically. For this purpose one set of each amino acid or their mixture was inoculated daily for a constant period of 15 days. On the 16th day the fungal mats from each set were separately filtered. The filtrate was chromatographically analysed while the growth records were taken by weighing the fungal mats of the 6th, 11th and the 16th days. The details of the chromatographic method were similar to those described by Ranjan *et al* (1955). Chromatograms were run on Whatman No. 1 filter paper, a 4 : 1 : 5 mixture of n-butanol : acetic acid : Water (BAH) was used as developing solvent. Chromatograms were sprayed with 0·1% n-inhydrin in n-butanol and were then dried at 65°C for 20 minutes. Visual observation was completed within an hour.

Experimental

TABLE 1

Dry weight and sporulation of *P. artocarpina* on media containing equivalent quantities of differet nitrogen compounds.

	Nitrogen compounds		Dry weight in mags.	Sporulation
1.	Potassium nitrate	...	95·3	Excellent
2.	Sodium nitrate	...	81·6	Good
3.	Calcium nitrate	...	72·3	Fair
4.	Magnesium nitrate	...	81·6	Good
5.	Sodium nitrite	...	0·0	...
6.	Potassium nitrite	...	0·0	...
7.	Acetamide	...	112·3	Poor
8.	Asparagin	...	99·6	Excellent
9.	Urea	...	68·6	Poor
10.	Thiourea	...	51·6	Poor
11.	Peptone	...	119·6	Good
12.	Ammonium chloride	...	44·6	Absent
13.	Ammonium nitrate	...	51·0	Absent
14.	Ammonium sulphate	...	42·6	Absent
15.	Ammonium carbonate	...	49·3	Absent
16.	Phenyl alanine	...	56·0	Poor
17.	Glycine	...	60·0	Good
18.	dl-valine	...	124·0	Poor
19.	Glutamic Acid	...	132·0	Fair
20.	Serine	...	62·0	Poor
21.	Tyrosine	...	66·3	Poor
22.	Histidine	...	46 3	Excellent
23.	Leucine	...	41·6	Poor
24.	Aspartic Acid	...	120·6	Fair
25.	Alanine	...	91·6	Fair
26.	Arginine	...	78·3	Fair
27.	No nitrogen	...	0·0	...
	Average	= 65·4		

Summary of dry weight results and conclusions at 1% level of P.

Treatments	...	highly significant
Replicates	...	non significant
S. E.	...	C. D. at 1% level
0·7.	...	2·61

Dry weight results

Nitrogen compounds-glutamic acid (132·0) > dl-valine (124·0) > aspartic acid (120 6), Peptone (119·6) >
acetamide (112·3) > asparagin (99·6) > potassium nitrate (95·3) > alanine (91·6) > mag. nitrate (81·6), sod. nitrate (81·6) >
arginine (78·3) > cal. nitrogen (72·3) > urea (68·6), tyrosine (66·3) > serine (62·0), glycine (60·0) >
phenyl alanine (56·0) > thiourea (51·6), amm. nitrate (51·0), amm. carbonate (49·3) >
histidine (46·3), amm. chloride (44·6), amm. sulphate (42·6), leucine (41·0) > sod. nitrite (0·0)
pot. nitrite (0·0), no nitrogen (0·0)

Assimilation rate of amino acids

The assimilation rate of those amino acids which were present in the host either in free form or in their protein complex was determined chromatographically. The following amino acid viz. glutamic acid (Rf 0·47) aspartic acid (Rf. 0·43), leucine (Rf. 0·80), histidine (Rf. 0·21), alanine (Rf. 0·53) and their mixture were used. The results are summarized in Table No. 2.

TABLE 2

Effect of different amino acids and their mixture in the medium and the dry weight of *P. artocarpina.*

Amino acids	Dry weight in mgs.			Presence of amino acid for the period ending
	6th day	11th day	16th day	
Glutamic acid ...	60·0	99·0	129·0	8th day
Aspartic acid ...	56·0	98·0	121·0	10th ,,
Leucine ...	17·0	30·0	40·0	16th ,,
Histidine ...	20·0	36·0	48·0	16th ,,
Alanine ...	39·0	68·0	89·0	12th ,,
Mixture of amino acids ...	68·0	107·0	134·0	11th ,,

Table 2 shows that the maximum mycelial growth of *P. artocarpina* was attained on a mixture of all the 5 amino acids. It is also clear from the table that good amino acid sources i. e. glutamic acid, aspartic acid and alanine were consumed earlier i. e. in 8, 10 and 12 days respectively while the poor sources viz. leucine and histidine were not finished even in 15 days. It was also noticed that all the amino acids included in the mixture were completely finished by 12th day even though individually some of them were not fully utilized upto 15th day. The chromatographic results indicated that both good and poor amino acids were utilized simultaneously from the mixture because a simultaneous decrease in the intensity of bands of all the amino acids was observed from 2nd to the 12th day.

DISCUSSION

The importance of nitrogen in the nutrition of *Phyllosticta artocarpina* was evident by the fact that the organism was in capable of growing in its absence. It was thus unable to assimilate the atmospheric nitrogen. Nitrites of sodium and potassium did not permit any growth and in this respect the results were similar to those of Ajello (1948), Gordon (1950), and Patel *et al* (1950). *Morchella esculenta* (Brock 1951), *Fusarium coeruleum* (Tandon and Agarwal, 1953), and *Hormodendrum resinae* (Morsden, 1954) could, however, grow on nitrites and thus they differed from *P. atrocarpina.*

The results from Table No. 1 showed that asparagin, acetamide, peptone, urea and nitrates of sodium, potassium, magnesium and calcium were significantly good sources. In this respect the results were similar to those obtained by the authors (1954 a) for *P. cycadina.* Out of the 11 amino acids used, dl-valine, aspartic acid, glutamic acid, alanine and arginine supported good growth and in this respect also the two organisms behaved in the same manner. Chromatographic analysis of the leaves of the host (*Artocarpus heterophyllus*) indicated that all these amino acids were available in the host in free form.

Thiourea, glycine, serine, phenylalanine, leucine and all the four ammonium compounds viz. ammonium nitrate, ammonium sulphate, ammonium carbonate and ammonium chloride were poor sources. Previous results of the authors (1954 a, b) had also shown these sources to be poor for *Phyllosticta cycadina.* Mix (1933) and Tandon and Grewal (1950) also reported that ammonium nitrogen was unsuitable for *Phyllosticta sitaria* and *Gloeosporium papayae* respectively. l-tyrosine was found to be only a moderate source for *P. artocarpina,* although it was a good source for *P. cycadina* (Tandon and Bilgrami, 1954.b). Histidine which was a good source for *P. cycadina* supported poor growth of *P. artocarpina.* This shows that inspite of great similarity in the nitrogen nutrition of *P. cycadina* and *P. artocarpina,* they differ considerably for the choice of some particular substance.

While studying the nutrition of wheat bunt fungus (*Tilletia caries*), Murey and Zscheile (1956) found evidence of antagonistic effects among various amino acids but in the present investigation maximum growth of *P. artocarpina* was attained on a mixture of amino acids. This was interesting because some of the constituents of the mixture viz. histidine, and leucine were individually unsatisfactory.

Little attention has so far been paid to the role of nitrogen nutrition on the sporulation of fungi. A critical survey of Table 1 makes it clear that there was no correlation between growth and sporulation and practically all grades of sporulation were recorded with significantly good or poor growth. In general peptone, asparagin, potassium nitrate, magnesium nitrate and sodium nitrate appear to be best for *Phyllosticta artocarpina* because these substances are suitable both for good growth and sporulation.

SUMMARY

The influence of 26 different sources of nitrogen was studied on the growth of *Phyllosticta artocarpina* (Syd *et Butl*). The organism belonged to group II of of Robbins's classification as it failed to assimilate atmospheric nitrogen. Nitrites inhibited the growth. Nitrates of inorganic salts, dl-valine, glutamic acid, aspartic acid, aspargin, arginine, peptone and urea were significantly good sources of nitrogen. Poor growth was recorded on ammonium compounds glycine, serine, leucine and histidine.

Chromatographic studies showed that there was no antagonstic effect of different amino acids and even the poor sources of amino acids did not adversely influence the growth when they were given in combination with good amino acids.

There was no correlation between growth and sporulation. It has been established that in general the nitrogen requirement of *P. artocarpina* is similar to that of *P. cycadina* except for comparatively poor growth on histidine and tyrosine.

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PRODUCTION OF HAPLOIDY IN EXPERIMENTALLY TREATED EGGS OF *GASTEROSTEUS ACULEATUS* (L)

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INTRODUCTION

During the course of production of polyploidy in *Gasterosteus aculeatus* (Swarup 1956, 1958) by temperature shock treatment of the eggs at the Zoological Laboratories, University of Oxford, it was observed that some of the embryos which developed from the treated eggs were haploid. Haploids are of interest in that they cannot be heterozygous and when a heterozygous form passes through such a haploid generation, the material so obtained is genetically pure.

Haploid embryos have been obtained from eggs of Echinoderms, Annelids, Molluscs and Ascidians though their development has not been followed beyond larval stages. Among Vertebrates the work on haploidy is mostly confined to Amphibia. It has been observed that the viability of the Amphibian haploid larvae is greatly reduced due to the retardation in growth and abnormalities of form. Even the 290 days old haploid larvae of *Triturus alpestris* died at metamorphosis (Fischberg 1944). Among the Pisces Svardson (1945) obtained three haploid embryos of *Salmo salar* by treating the eggs of *Salmos alar* with sperm of *Salmo trutta* and subjecting the eggs to a cold shock. These haploid embryos were squashed at cleavage stages for chromosome analysis and their further development was not studied by Svardson.

In the present paper an account has been given of the haploids produced by temperature shock treatment of the eggs of *G. aculeatus* and an analysis has been made of the possible mechanism which is responsible for its production.

MATERIAL AND METHOD

Gasterosteus aculeatus is a fresh water fish, which is quite common in Great Britain, It is a small fish rarely exceeding 55 mm. in length and its span of life is about a year. It normally breeds during the months of April and May and lays 100 to 150 eggs at a time. The eggs measure 1·5 to 1·7 mm. in diameter and the embryos hatch out in 6-8 days at a temperature of 18°-19°C.

The haploids recorded in the present paper were produced as by-product in the experiments conducted for the production of artficial polyploidy by temperature shock for which eggs were stripped from ripe females and treated with teased testes from mature males. Control experiments were also made to produce artificially induced haploidy by temperature shock treatment of the eggs without treating them with mature testes material.

Chromosome analysis was made from aceto-orcein squash preparations of the embryos.

OBSERVATIONS

The factors applied to eggs freshly treated with teased testes were as follows:

Hot-shock: Eggs after treatment with testes material for 3,5, 10, 15 and 20 minutes were treated for 5 minutes at temperatures of 32·5, 33·0, 33·5 and 34·0°C. A temperature of 34·0°C or above was fatal for the eggs.

Cold-shock: Eggs after treatment with testes material for 3, 5, 10 and 15 minutes were treated for ½, 1, 1½, 2, 2½ and 3 hours at a temperature of 0°C. A duration of more than 3 hours was fatal to the eggs.

The conditions under which the best results were obtained are given in Table I, which also gives percentage of haploids produced.

TABLE I

Showing production of haploids under optimum conditions of Hot and Cold Shock treatment.

Treatment	Total eggs treated	Survival upto eye pigmentation	Analysable	Diploid	Haploid No.	Haploid %	Other chromosome numbers
33·5-34·0°C for 5 minutes, 15 minutes after insemination	168	49	37	7	3	8·1%	27
0°C for 3 hours, 15 minutes after insemination	85	45	38	14	17	44·8%	6

From the above table it is seen that cold shock produces more haploids (44·8 of the analysable eggs) than does the hot shock (8·1% of the analysable eggs). The haploids produced by both cold and hot shock treatment reached a stage with well developed brain and eyes and with pigment in the eyes as well as in the skin. These haploid embryos were, however, weak and slow in development as compared with the normal diploids. Moreover the percentage of abnormal haploid embryos produced was very great in the case of those produced by hot shock as compared to those produced by cold shock. The viability of the haploids could not be studied up to the time of hatching as all the embryos obtained were squashed for chromosome analysis.

Since there was a possibility of some of the eggs escaping fertilization and being activated by temperature treatment it was considered worthwhile to perform control experiments for parthenogenetic production of embryos. Therefore eggs stripped from ripe females and without treating them with testes material were given similar temperature treatments as those which produced haploids. The results obtained are given in Table II.

TABLE II

Results obtained by treating eggs not treated with testes material by Hot and Cold Shock treatment.

Treatment	Total No. of eggs treated	Eggs showing activation	Eggs showing segmentation
33·5-34·0°C for 5 minutes.	37	9	2
0°C for 3 hours.	137	88	12

It is interesting to note that some of the unfertilized eggs show activation and a very small number even show cleavage divisions leading to blastula stage but none of them reach the gastrulation stage. It is therefore most unlikely that the haploid forms shown in Table I were produced parthenogenetically.

DISCUSSION

The production of haploidy by temperature shock treatment of eggs treated with spermatic fluid has been observed in Salmon (Svardson 1945), Amphibia (Fankhauser 1945 and Fischberg 1948) and mouse (Fischberg and Beatty 1952). So far it is only in Amphibia that artificially produced haploids have been reared after hatching, but even here they could not survive metamorphosis. In Salmon as well as in mouse the haploids are known only from eggs showing cleavage stage. It is therefore for the first time that in fish artificially produced haploids have reached a high stage of embryonic development, almost up to the hatching stage.

The viability of the fish haploids is much reduced as in the case of haploid Amphibians. Various hypotheses have been proposed to account for the poor viability of these individuals. It has been suggested (Humphrey 1943) that lethal factors may be present in most of the chromosome sets and exert their effect at various early stages of development unchecked by the normal dominant alleles usually contained in the second set of chromosomes in a diploid. It is also suggested (Brachet 1944, 1947) that the biochemistry of the haploid cells is disturbed because of some deficiency in the synthesis of ribose nucleoprotein. According to Fankhauser (1956) lack of heterochromatin in the nuclear material or an upsetting of gene dosage in the haploids may be factors connected with such reduced viability. Observations of Briggs (1947) on haploid frog embryos developed from large and small eggs support the thesis that it is the initial disturbance of the nucleo-cytoplasmic ratio which is responsible for reduced viability of the haploid embryos. In his experiments a reduction of the initial amount of cytoplasm and yolk resulted in more normal development of the haploid frog embroyos. It is, however, not possible at this stage of our knowledge to assign one or more causes with any certainty.

As regards the mechanism which produces haploidy, it may be either gynogenesis or androgenesis. Gynogenetic haploids have been produced in Amphibia

(Dalcq 1929, Rugh 1939, Fischberg and Selman 1952) and mouse (Edwards 1954) by fertilizing the eggs with spermatozoa which were previously inactivated genetically by treating them with certain chemicals like trypaflavine, toluidine blue etc. or by x-rays or ultra-violet rays. In the present work, as the eggs were treated with testes material containing active spermatozoa it is unlikely that the sperms would degenerate inside the egg. On the other hand it is more likely that the abnormal temperature effected the egg nucleus which would be in its meiotic phase. It has been shown by Kaylor (1940) and Hamilton (1957) that in Amphibians if the egg nucleus is inactivated or removed after fertilization the haploid sperm nucleus alone takes part in development. Thus it is quite possible that in *G. aculeatus* also the haploid fish embryos obtained by temperature shock treatments have been produced by androgenesis.

It is important to point out that in many cases of the so-called parthenogenetic development in Fishes (Lestage 1934, Kasansky 1935) and Amphibians (Bataillon 1910, Parmenter 1933 and Kawamura 1939) which reached advanced stages and even sexual maturity, it has been shown that such forms contained diploid chromosome numbers. This diploidy might have occured either by the fusion of haploid nuclei at the first or a later cleavage division or by the fusion of the haploid egg nucleus with the nucleus of the polar body. In *G. aculeatus*, however, no diploid forms were produced under the conditions of experiments given in Table II.

SUMMARY

1. Haploidy has been produced in *Gasterosteus aculeatus* by subjecting the eggs treated with teased testes to temperature shock treatments. Hot Shock treatment of 33·5°-34·0°C for 5 minutes, 15 minutes after treatment with testes material produces 8·1% haploidy while Cold Shock treatment of 0°C for a duration of 3 hours, 15 minutes after treatment with testes material produces 44·8% haploidy.

2. The haploids produced reached an advanced embryonic stage showing well developed brain, eyes and pigmentation in the skin.

3. The viability of these haploids seems to be much reduced as most of the embryos were weak and slow in their development.

4. Temperature shock treatment initiates parthenogenetic development in the unfertilized eggs also but in all such cases the eggs fail to develop beyond the blastula stage.

5. It is suggested that the haploids have been produced due to androgenesis.

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THE DEVELOPMENT OF SUPRABRANCHIAL ORGAN AND THE ASSOCIATED BLOOD VESSELS IN *OPHICEPHALUS PUNCTATUS* (BLOCH)

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INTRODUCTION

Ophicephalus punctatus is a fresh water fish of tropical countries. It grows to about nine inches to a foot in length and inhabits shallow stagnant waters of pools and ditches where there is luxurient growth of aquatic vegetation. This fish possesses an accessory respiratory organ and hence is able to utilise directly the atmospheric oxygen for respiratory purposes. The accessory respiratory organ consists of a pair of pouches, the suprabranchial organ, in the roof of the pharynx. The fish comes frequently to the surface of water to gulp fresh air through the mouth. The air from the mouth passes into the suprabranchial organ and then passes out through the gill opening.

The first account of the suprabranchial organ in *O. punctatus* was given by Hyrtl (1853) who described them as branchial chambers meant for the storage of water to moisten the gill filaments. The true nature of these chambers or cavities was pointed out by Day (1868) and later by Dobson (1874). They concluded that these cavities contained air for purposes of direct or aerial respiration and thus behave as accessory respiratory organs. Since then these organs have been studied by various authors, the important contributions in the field being those of Das (1928), Lele (1932), Bader (1937) and Wu and Hsiao-Weichang (1946). These accounts are based on the physiology of the organ rather than on structural modifications and deal mainly with such structures as the mucous lining and the blood supply of the organ in adult fishes. A more detailed account of the blood circulation of the suprabranchial organ in *O. striatus* has been recently given by Das and Saxena (1956). None of these authors, however, have studied the blood circulation in embryonic condition and, therefore, were unable to trace the modification of the blood vessels from their normal condition.

MATERIAL AND METHOD

The early embryonic stages and the adult specimens of *O. punctatus* were obtained from Saugar Lake, Sagar. Serial sections of various stages were stained with Mallory's triple stain. Skull preparations of adult specimens were also made.

OBSERVATIONS

The first indication of the suprabranchial organ is seen at 8 mm. stage (fig. 1). The pharyngeal roof shows a thickening of the mucous lining in the region of the first and second gills just behind the pseudobranch. Moreover, a shallow depression is seen on either side of the pharyngeal roof above the first gill. By 12 mm. stage

(fig. 2), these depressions have sunk deeper to form pouch like structures, the pharyngeal pouches, which open in the pharynx by wide apertures; the walls of these pouches have become thicker and infolded. By this time, blood spaces have also appeared in the walls and the surrounding connective tissue of the pouches. As development proceeds further, the pouches grow in size and extend backwards as far as the fourth pair of gills. The wide openings of the pouches in the pharynx as seen at 12 mm. stage, become narrower by the approximation of its edges and the backward extension of the pouches take on the form of posteriorly directed processes. At 15 mm. stage (fig. 3), the suprabranchial organs show well developed infoldings of the wall and their blood supply is also fully established

The blood vessels of the head region in *O. punctatus* have undergone profound modifications because of the presence of the suprabranchial organ and pseudobranch. At 5 mm stage (fig. 4), the median dorsal aorta divides into two internal carotid arteries (paired dorsal aortae) at the hind end of the skull and these run forward parallel to each other just beneath the parachordals. On their way the carotid arteries cross the prootic bridge of the chondrocranium and enter the hypophysial fenestra. Here each carotid artery crosses the trabecular cartilage of its side dorsally and is continued forward parallel to the latter. During its course it gives out an optic artery to the eye, a posterior and an anterior cerebral artery to the brain and then becomes continued forward as the orbitonasal artery, which enters the nasal capsule. Besides the arteries mentioned above, each carotid artery is associated with the arteries coming from the four gills and the pseudo branch of its side. The four gills of each side receive venous blood from the median ventral aorta through the four afferent branchial arteries. After oxygenation the blood from the gills of each side is sent to the four efferent branchial arteries. Each efferent branchial artery arises from its gill lateral to the branchial arch and moves upwards and inwards to meet the carotid artery of its side. On each side just behind the eye, but anterior to the first gills there is a pseudobranch embedded in the connective tissue. The afferent blood vessel of each pseudobranch is connected to the carotid artery of its side through the orbital artery at a point where it enters the hypophysial fenestra. Distally, each orbital artery gives out a mandibular artery to the lower jaw. The afferent blood vessel of the pseudobranch runs towards the carotid and just close to it takes a sharp turn anteriorly and becomes an ophthalmicamagna artery going to the eye. The venous blood from the head is collected by a pair of head veins (anterior cardinals) which come out of the trigeminofacialis chamber through the facial foramen and run posteriorly beneath the auditory capsules to join the heart.

The arrangement of blood vessels at 5 mm. stage is what should be expected with the exception of the blood supply to the pseudobranchs which receive oxygeneted blood from the carotids. Later with the appearance of the pharyngeal pouches at 8 mm. stage the blood vessels in their region become much modified (fig. 5). The first pair of afferent branchial arteries lose their connection with the carotids and become the afferent vessels of the suprabranchial organ. They subdivide into a set of capillary network which surrounds the connective tissue of the pouches. The second pair of efferent branchial arteries behave in the same manner but at this stage they have not completely severed their connection and show a weak link with the carotids. At the same time a new set of blood capillaries arise in the connective tissue of the pouches and they soon establish their connection with the head veins.

At 15 mm. stage the modification of the blood vessels in relation to the suprabranchial organ is complete. The afferent and efferent branchial arteries of the first gill develop direct connection with one another so that the blood from the

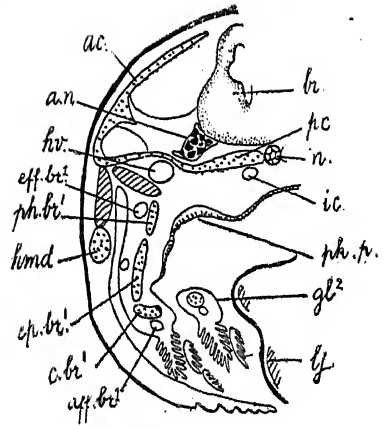

Fig. 1. T. S. 8 mm. Stage of *Ophicephalus punctatus*: showing the depression in the pharyngeal roof where the pharyngeal pouch would develop ac. = auditory capsule; aff. = afferent branchial artery; an. = auditory nerve; br. = brain; c. br. = cerato-branchial cartilage; eff. br. = efferent branchial artery; ep. br. = epibranchial cartilage; gl. = gill; hmd. = hyomandibular cartilage; hv. = head vein; ic. internal carotid artery; lj. = lower jaw; n. = notochord; pc. = parachordal cartilage; ph. br. pharyngobranchial cartilage; ph p. = pharyngeal pouch.

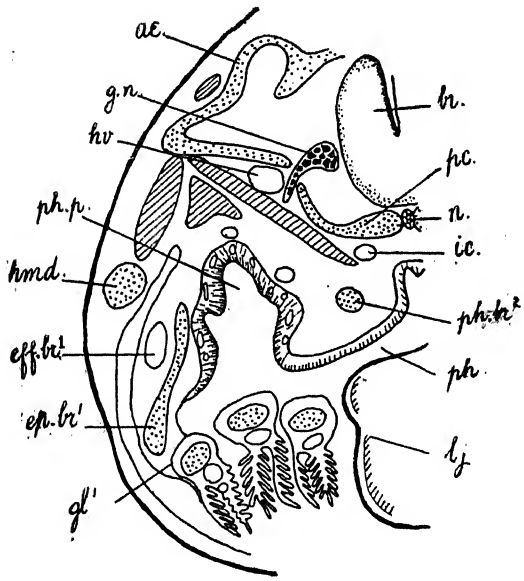

Fig. 2. T. S. 12 mm. Stage of *Opnicephatus punctatus* showing the pharyngeal pouches opening in the pharynx; ph. = pharynx; rest letterings as in fig. 1.

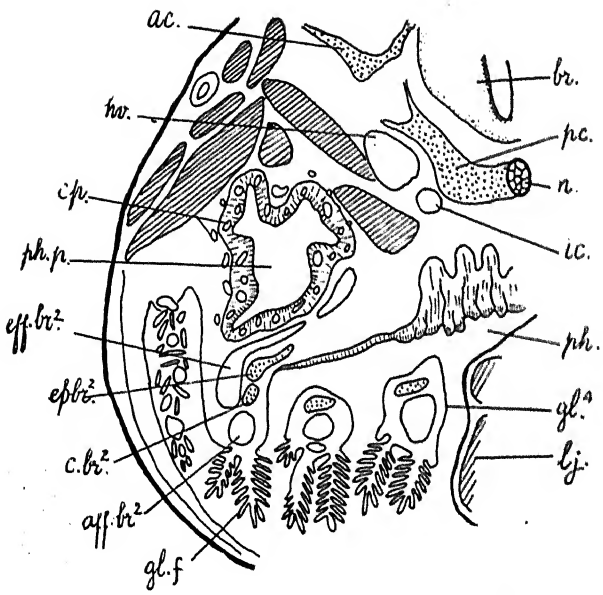

Fig. 3. T. S. 15 mm. Stage of *Ophicephalus punctatus*, showing the pharyngeal pouch in the posterior region ; letterings as in fig. 1.

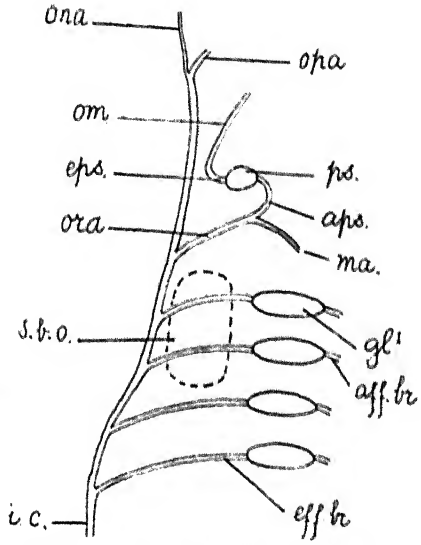

Fig. 4. Diagrammatic view of the anterior arteries of the right side of a 5 mm. Stage of *Ophicephalus punctatus* : aps = afferent pseudobranchial artery ; eps. = efferent pseudobranchial artery ; ma. = mandibular artery ; o.m. = ophthalmicamagna artery ; ona. = orbitonasal artery ; opa. = optic artery : ora. = orbital artery ; ps. = pseudobranch ; sbo. = suprabranchial organ : rest letterings as in fig. 1.

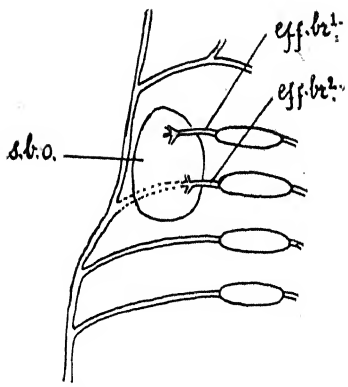

Fig. 5. Diagrammatic view of the branchial arteries of the right side of a 8 mm. Stage of *Ophicephalus punctatus* : letterings as in fig. 4.

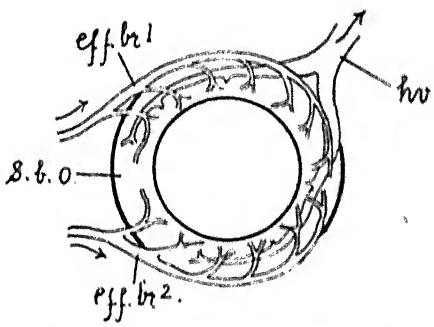

Fig. 6. Diagrammatic view of the blood vessels of the supra branchial organ of a 15 mm. Stage of *Ophicephalus punctatus* : letterings as in fig. 1 and 4.

afferent artery can pass either directly to the efferent artery or thr ough the capillary network in the gill filaments. The second afferent and efferent branchial artertes are also similarly modified. Thus, during aerial respiration the blood from the first and second afferent arteries can pass into the corresponding efferent arteries directly while during aquatic respiration, through the branchial capillary system. The blood capillaries of the connective tissue surrounding the suprabranchial pouch, have now invaded even the epithelial lining of the pouches. The relationship of these blood vessels to the suprabranchial organ are shown diagrammatically in figure 6. The first efferent branchial vessel supplies bood to the anterior and dorsal part of the pouch while the second supplies blood to the posterior and ventral part. The head vein lies dorsal and median to the pouch.

Thus when the fish is breathing air, the blood from the ventral aorta is passed on directly to the suprabranchial organ through the first and second pair of afferent and efferent branchial vessels which form complete loops. The oxygenated blood from the suprabranchial organ is sent to the heart through the head veins. It is evident that the blood which has been oxygenated in the suprabranchial organ has to go from the heart to the third and fourth pair of gills before it is sent to the dorsal aorta for general distribution.

DICUSSION

The experimental work of Das (1928) proves beyond doubt the air breathing function of the suprabranchial organ but his account of blood circulation is vague. The accounts of the circulation of blood in *O. punctatus* by Lele (1932) and that in *O. striatus* by Das and Saxena (1956) are clear but these authors deal only with the blood supply in the adult in which the suprabranchial organ is fully developed. None of the previous authors, however, have made a study of the blood vessels during development. The present study shows for the first time the embryonic condition of the blood vessels and the changes undergone by them during development. The first and second efferent branchial arteries which are connected with the carotids in the embryo, lose their connection in the adult and send the blood to the suprabranchial organ. It is interesting to note that the first efferent branchial artery lies dorsal to the organ while the second lies ventral to it. This difference in position of the two arteries can be explained as follows—the pharyngeal pouches first appear as small depressions in the region of the first gill; but they soon grow deeper and push the first efferent branchial arteries dorsally, which under the strain of the developing pouches, break their connection with the carotids. In their second phase of development the pouches extend backward. The posterior processes of the pouches pierce through the connective tissue of the pharyngeal roof in the region of the second gill and in so doing come to lie dorsal to the second efferent branchial arteries. It is in this manner that the second pair of arteries are late in breaking connection with the carotids and lie ventral to the suprabranchial organ.

SUMMARY

1. The development of the suprabranchial ogran in *O. punctatus* is described. It arises in the form of paired depressions in the roof of the pharynx at 8 mm. stage. These depressions sink deeper and become pouches at 12 mm. stage. The mucous lining of the pharynx in the region of the pouches becomes thickened, infolded and highly vascular. At 15 mm. stage the pouches have grown in extent by projecting backwards; their opening into the pharynx narrows and they begin functioning as accessory respiratory organs.

The blood vessels which are associated with the suprabranchial organ are greatly modified. The first and second efferent branchial arteries which are well developed at 5 mm. stage become weak and break their connection with the internal carotids at 8 mm. stage. In a fully developed suprabranchial organ at 15 mm. stage, the first and second afferent branchial arteries become continuous with the corresponding efferent branchial arteries so that during aerial respiration the blood may pass from the ventral aorta to the suprabranchial organ directly without having to pass through the branchial capillary system.

ACKNOWLEDGMENT

The author is grateful to Professor D. S. Srivastava for his guidance and interest in the preparation of this paper.

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THE MODIFICATION OF THE BONES OF THE SKULL IN RELATION TO THE SUPRABRANCHIAL ORGAN IN *OPHICEPHALUS PUNCTATUS* (BLOCH)

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(Received on December 29th, 1957)

INTRODUCTION

The development of the suprabranchial organ in *Ophicephalus punctatus* for the purpose of aerial respiration, has already been described by the author (Swarup 1958). The presence of this organ in the roof of the pharynx greatly modifies the ossification of the bones of the skull in that region. These modifications of the skull bones have not been described by anybody so far. The present author has already described the development and morphology of the skull in *O. punctatus* (Swarup 1954, 1955 and 1956) and the present paper deals with the modifications of the bones of the skull in relation to the suprabranchial organ.

MATERIAL AND METHOD

The skull preparations were made of specimens obtained from the Saugar Lake, Sagar (Madhya Pradesh). Transverse sections of the head of earlier stages were also prepared.

OBSERVATION

The suprabranchial organ is well developed at 15 mm. stage (Swarup 1958), when the cartilages of the chondrocranium are fully formed. It lies ventral to the auditory and occipital regions in between the hyoid and the first branchial arch and is bounded laterally by the hyosymplectic, ventrally by the first epibranchial and medially by the pharyngobranchial cartilages. The ossification of the cartilages and the appearance of the dermal bones take place early and continue till 30 mm. stage when all the skull bones are completely formed. The bones described here belong to an adult fish.

The skull shows ventrally at the hind end a pair of cup like depressions known as the subtemporal cavities. These cavities in association with the hyomandibular, first epibranchial and fourth and fifth pharyngobranchial bones form a complete support to the folds of the mucous lining of the suprabranchial organ (Figs. 1 & 2).

Each subtemporal cavity is formed of six cranial bones : the pro-otic, pterotic, epiotic, exoccipital, supraoccipital and parietal (Fig. 3).

The pro-otic bone occupies the anterior and median edges of the cavity. It is a thick irregular bone, largest of the series and forms the entire auditory capsule. The pterotic which occupies a lateral position is an elongated flat bone and extends dorsally inwards to form a part of the dorsal surface of the cavity. It takes no part

in the formation of the auditory capsule and shows an elongated groove on its ventral surface for the articulation of the hyomandibular bone. The posterior part of the cavity is formed by the epiotic bone which consists of a thick cup like structure and is far removed from the pro-otic bone. The exoccipital bone which lies lateral to the occipital region and ventral to the supraoccipital and epiotic bones forms the postero-ventral edge of the cavity. The supraoccipital is a median bone and contributes to the formation of the medial surfaces of both the cavities. It is a thick symmetrical bone and occupies the dorsal occipital region coming in between the two parietal bones. Laterally the supraoccipital shows cavities which are wedged in between the pro-otics and epiotics. All these five bones are arranged in a circle and slope ventrally inwards in a manner so as to enclose the subtemporal cavity of each side. The inner margins of these bones are strengthened by the superimposition of the parietal bone on the dorsal surface.

The hyomandibular bone articulates dorsally with the pterotic and forms the lateral wall of each suprabranchial cavity. From the inner surface of hyomandibular a median process is given out (Fig. 4) which hangs into the cavity. This process provides extra surface for the folds of the mucous membrane.

The epibranchial bone of the first branchial arch has become much expanded (Fig. 5) and is situated ventral to each subtemporal cavity. It not only carries a fold of the mucous lining but is movable and acts as a valve for the passage of the air.

The pharyngobranchial bones of the fourth and fifth branchial arches are more or less united and form a flat bone which forms the ventral support of each cavity behind the first epibranchial.

Thus the presence of the suprabranchial organ has resulted in the modification of the bones of the skull which is changed considerably in shape in the hind region. The effect of these modifications can be seen in the following points :

1. Presence of subtemporal cavities.

2. Shifting of the auditory capsules towards the medial side.

3. Enlargement of the prootics which form the entire auditory region.

4. Loss of share of other otic bones (sphenotics pterotics and epiotics) to form the auditory capsules.

5. Forward extention of the supraoccipital to come in between the two parietals.

6. Lateral shifting of the parietals to form the dorsal covering of the temporal cavities.

7. Cup like formations on the lateral sides of the supraoccipital and anterior side of the epiotic.

8. Flattening of the hyomandibular and the development of the hyomandibular process.

9. Expansion of the first epibranchial.

10. Fusion and flattening of the fourth and fifth pharyngobranchials.

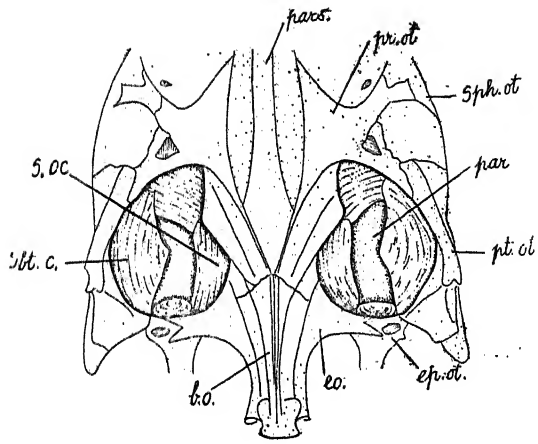

Figure 1. Ventral view of the hind part of the skull of *O. punctatus* showing the subtemporal cavities. bo =basioccipital bone, eo =exoccipital bone, ep. ot-epiotic bone, pars =parasphenoid bone, par =parietal bone, pr. ot =prootic bone, pt. ot =pterotic bone, sbt. c =subtemporal cavity, sph. ot =sphenotic bone, soc =supraoccipital bone. Mag. 5 X.

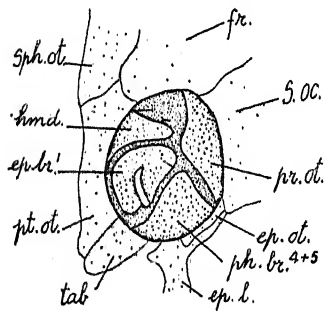

Figure 2. Dorsal view of the hind part of the left side skull of *O. punctatus* after the removal of the parietal and other bones attached to it to show the subtemporal cavity from the dorsal side. ep. 1 =epiotic lamilla fr=frontal bone, tab =tabulare bone. Mag. 5 X.

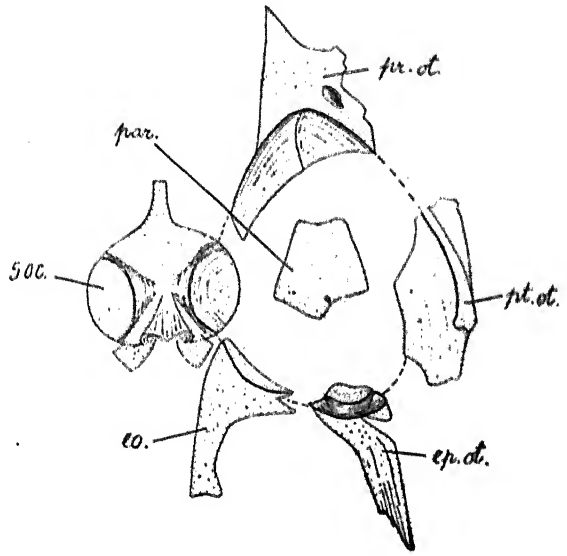

Figure 3. Disarticulated bones of the skull of *O. punctatus* showing the arrangement of the six bones which form the subtemporal cavity. Mag. 5 X.

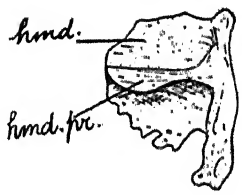

Figure 4. Hyomandibular bone showing the expanded nature and its process. hmd. pr=hyomandibular process. Mag. 5 X.

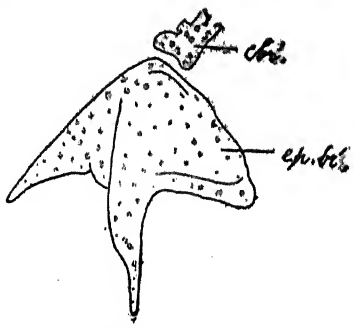

Figure 5. First epibranchial bone showing the expanded nature. Mag. 5 X.

DISCUSSION

The earlier accounts of the skull of *O. striatus*, another genus of the family Ophiocephalidae to which *O. punctatus* also belongs, make no mention (Day, 1914; Bhimachar, 1932) about the modifications of the skull due to the presence of the suprabranchial organ in that fish. Lele (1932) did, however, notice the compressed nature of the auditory capsules caused by the growing air chambers in their region. The recent account of the skull of *O. punctatus* by Swarup (1956) also does not refer to these modifications. Therefore, the modifications of the bones described in the present paper are significant.

It is evident that the chondrocranium is fully formed at 8 mm. stage (Swarup, 1954) when the suprabranchial organ first shows its appearance and hence the cartilages show no change. The suprabranchial organ is completely formed at 15 mm. stage (Swarup, 1958) by which time the ossification of bones has not yet started. When the ossification does set in at the later stage, it has to meet the requirements created by the presence of the suprabranchial organ. Thus the modifications of the skull bones as seen in the hinder region are secondary in nature.

SUMMARY

The suprabranchial organ effects the ossification of the associated skull bones. The bones effected are pro-otics, pterotics, epiotics, exoccipitals, supraoccipital parietals, hyomandibulars, first epibranchials, and fourth and fifth pharyngobranchials. The change in shape of the skull is seen in the presence of subtemporal cavities, pro-otics forming the entire auditory capsules which become shifted medially, the separation of the two parietals by the median supraoccipital, the development of the hyomandibular process and the flattening of the first epibranchial and fourth and fifth pharyngobranchials.

ACKNOWLEDGEMENT

The author is grateful to Professor D. S. Srivastava for his guidance and interest in the preparation of this paper.

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ON THE TREMATODE—PARYPHOSTOMUM MEHRAI FARUQUI

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(Communicated by Dr. H. R. Mehra)

(Received on 10th December, 1957)

The trematode reported below was obtained from the intestine of white rat by feeding echinostome encysted cercariae of the species *Cercaria mehrai* Faruqui from the snail *Indoplanorbis exustus*. Observations on all its life-cycle were made. It was experimentally shown that the miracidium develops directly into a mother-redia, which afterwards produces a generation of daughter rediae and these in turn produce the cercariae. The entire process took eight weeks from the entry of the miracidium until the cercariae were matured. The mammalian phase of the life-cycle involves the feeding of cysts of these cercariae to the final host. The metacercariae develop into the adults in the intestine of the host in twenty days when the adults begin to lay their eggs, which are passed out with the faeces of the host. The adults thus obtained belong to the genus *Paryphostomum* (Dietz).

Paryphostomum mehrai Faruqui

The adults are elongated, flat, and are conical in the pre-acetabular region while behind it the sides of the body are almost rectilinear. The posterior end is almost rounded. Mature and well flattened specimens (Fig. 1) measure from 8·36 mm. in length and 1·6 mm. to 0·96 mm. in breadth at the acetabular region. At the anterior extremity the body forms an arched shape collar which is strongly developed. The collar (Fig. 2) is armed with 43 spines arranged in two rows uninterupted dorsally. The spines in the terminal group are a little larger than those in the marginal ones.

The acetabulum is well developed, muscular and measure 0·96 mm. ×0·83 mm. in diameter, with a roughly oval outline. The length of the acetabulum is about 1/11 of the body length for a specimen 9·8 mm. in size.

The oral sucker is small, rounded and subterminal measuring 0·247 mm. in diameter. The shape of the opening is usually oval. The ratio of the diameter of the oral sucker to the ventral sucker is 1:3·5.

The mouth opens into the cavity of the oral sucker followed by a distinct prepharynx 0·069 mm. in length. The pharynx (Fig. 3) is usually oval in shape and measure 0·24 mm. in diameter. The oesophagus is short and measure 0·22 mm. in length. The intestinal bifurcation occurs at about a distance of 0·256 mm. in front of the ventral sucker, and pass out widely round the ventral sucker and terminate at about 0·43 mm. from the posterior end of the body. The average diameter of the caecum is 0·09 mm.

The excretory bladder is of the usual type found in the Echinostomes. The excretory pore is situated on the dorsal surface at about 0·76 mm. distance in front

of the hinder end. The excretory bladder is Y-shapped with a short elongated vesicle like median stem produced in front into two very long cornua, which extend anteriorly up to the pharynx. The median vesicle of the cornua are produced into many irregular lateral branches outside, which are further branched. All along their course, the main stem and cornua send their diverticula towards the edge of the body.

The male and female ducts open separately. The male opening lies median close in front of the female opening a little in front of the acetabulum behind it and the intestinal bifurcation.

The testes (Fig. 1) are distinctly lobed and situated median close together in the median line, directly behind one another approximately half way between ventral sucker and hinder end of the body. There are five lobes in the anterior testis of which one is deeply intended. The posterior testis consists of six lobes. The post-testicular space measures 4·8 mm. in a specimen of 9·8 mm. length. The cirrus-sac (Fig. 1) 1·98 mm. in length and 0·384 mm. in breadth is elongated with the narrow end directed forward with a slight inclination ventrally on the ventral sucker. The swollen basal end extends behind the posterior margin of the latter. The vesicula-seminalis is also elongated, filling the greater portion of the cirrus-sac measuring 0·994 mm. × 0·552 mm. in size. It leads into a wide elongated duct of 0·864 mm. in length which runs backward within the pouch along the left margin of the vesicula-seminalis almost up to the level of the posterior margin of the acetabulum. It then leads forward in the form of a V-shaped tube running along the side of the right margin of the cirrus-sac. It opens anteriorly into pars-prostatica. The prostatic-duct runs forward to enter the ejaculatory duct. The pear-shaped prostatic cells form a mass surrounding the pars-prostatica into which they open by their narrow ductules.

The ovary is situated a little to the right side and is pre-testicular. It is nearly pear-shaped with an entire margin and measures 0·44 mm. in diameter. The shell gland mass lies a little posterior to ovary and occupies a median position. The oviduct passes backwards to the left-side and bulges into a sac like structure just before it's junction with the receptaculum seminis on one side and Laurer's canal on the other. The ootype soon after leaving the Laurer's canal narrows into a fairly long tube and passes to the left side of the shell gland mass, and receives the narrow common yolk duct of the vitelline reservoir.

The vitellaria occupy the lateral margins of the body extending from the posterior border of the ventral sucker to the hinder end. They overlap the intestinal caeca in the testicular region and practically unite with one another immediately behind the testes. The vitelline follicles are pear-shaped and do not overlap the ovary and the shell gland mass.

The uterus arises from the right side of the ootype and has some what muscular walls. It soon passes ventrally and then forwards as a wide thin walled tube which soon becomes coiled in somewhat S-shaped manner, and reaches the level of the posterior end of the ventral sucker where it opens into the metraterm. The metraterm proceeds forward to open to the exterior at the female genital aperture situated slightly posterior to the male aperture. The ova are numerous, thin shelled, bright yellow in colour and measures 0·096 mm. × 0·064 mm. in size.

DISCUSSION

The parasite, having stout, leaf-like body narrow anteriorly, broad and rounded posteriorly with deeply lobed testes, cirrus-sac lying lateral to acetabulum extending posterior to it, confluent vitellaria in the post-testicular region, belong to the genus

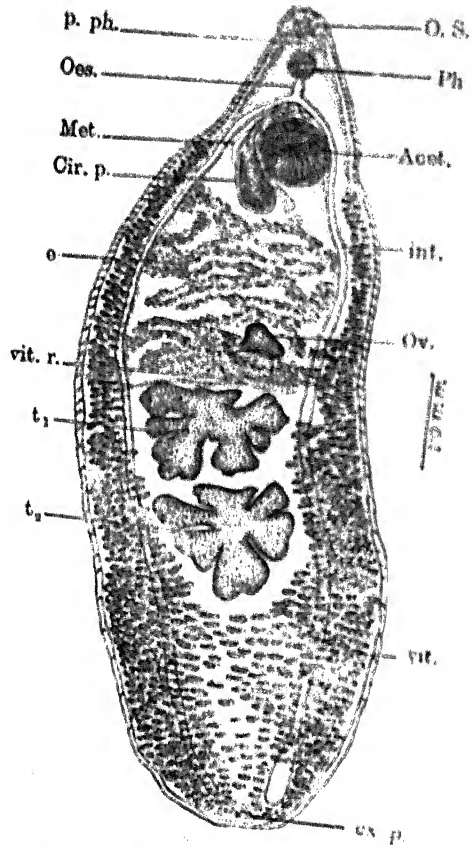

Fig. 1—Dorsal view of flattened *P. Mehrai*.

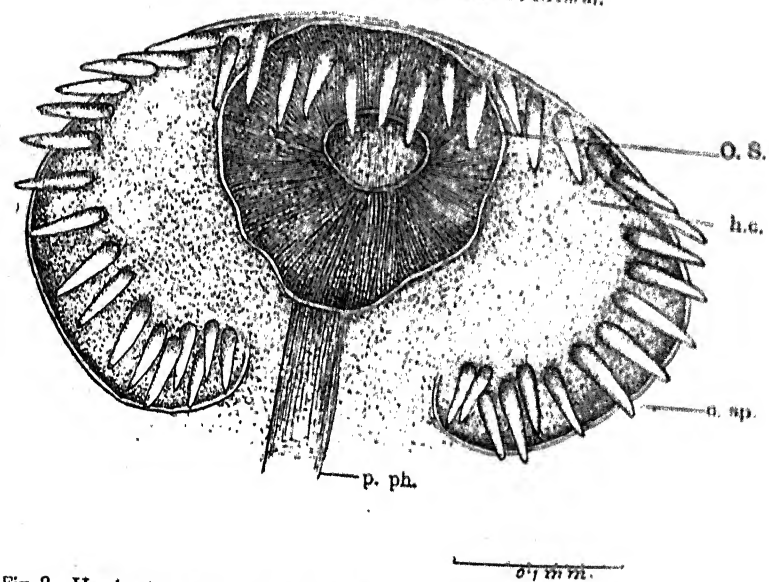

Fig. 2—Head collar showing the number and arrangement of collar spines.

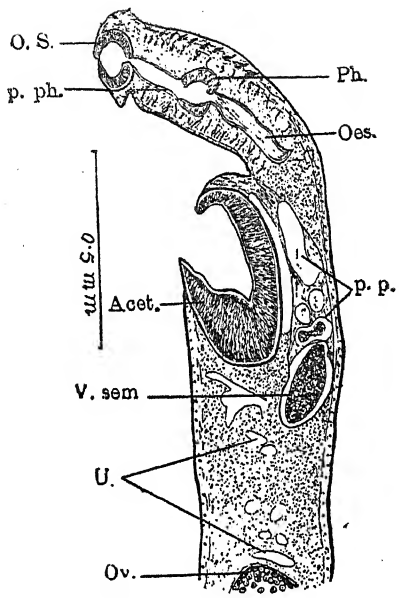

Fig. 3—Sagittal section through anterior half of body.

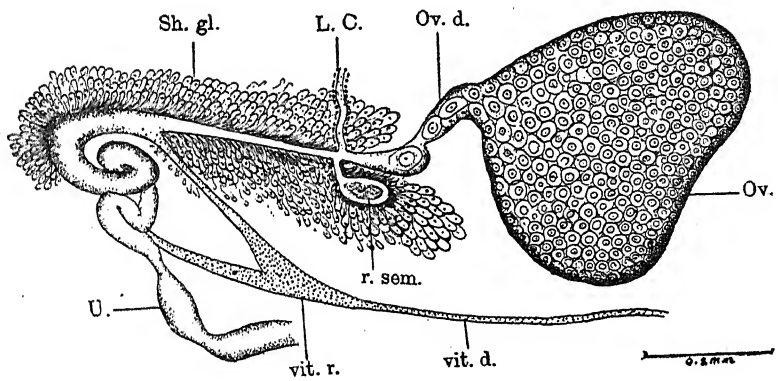

Fig. 4—Side view of the shell gland complex.

Lettering to Fig. 1-4.

Acet. Acetabulum; Cir. p. Cirrus pouch; C. sp. Collar spines; ex. p. Excretory pore; e. Eggs; h. c. Head collar; int. Intestinal caeca; L. C. Laurer's canal; Met. Metraterm; ov. Ovary; Oes. Oesophagus; O. S. Oral sucker; ov. d. Oviduct; Ph. Pharynx; p. ph. Prepharynx; p. p. Parsprostatica; r. sem. Recepticulum seminis: Sh. gl. Shellgland mass; t. Testes; U. uterus.

Paryphostomum Dietz, 1910. The nine species so far described (excluding *P. novum* Verma, 1936) have been included in this genus, viz. *P. radiatum* (Dujardin, 1845), Dietz, 1910; Edward, 1927; *P. segregatum* Dietz, 1910; *P. sufrartyfex* (Lane, 1915) Bhalerao, 1931; *P. testitrifolium*, Gogate, 1934; *P. indicum*, Bhalerao, 1931; *P. tenucollis* (Johnston, 1917) Price, 1931; *P. pentalobum* Verma, 1936; *P. phalacrocoracis* Goss, 1940; and *P. horai* Baugh, 1949.

The presence of 43 spines arranged in double row and the cirrus sac extending beyond the posterior margin of the ventral sucker distinguishes the present species *P. mehrai* (Faruqui) from *radiatum, segregatum, testitrifolium, pentalobum,* and *horai* but *sufrartyfex* and *indicum*. In the larger number of the spines and elongated cirrus sac, it resembles *P. testitrifolium* and *P. indicum*, but differs from them in having prepharynx, receptaculum seminis and absence of cirrus. It can be segregated from *P. indicum* where the cuticle spines are pre-ovarian and vitelline follicles commence from the middle of ventral sucker. The presence of a large number of eggs in the uterus is very characteristic of the new species.

ACKNOWLEDGEMENT

I have great pleasure to acknowledge my gratitude for the advice and help to Dr. H. R. Mehra under whose guidance this work was done. I also take this opportunity of expressing my sincere thanks to the U. P. Government for the grant of a Research-Assistantship under Dr. H. R. Mehra.

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